Detection and Identification of Metabolites from *Desulfovibrio Vulgaris Hildenborough* Lysate by Capillary Electrophoresis-Electrospray Ionization-Time of Flight-Mass Spectrometry

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT — OFFICE OF SCIENCE, U.S. DEPARTMENT OF ENERGY

VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov/

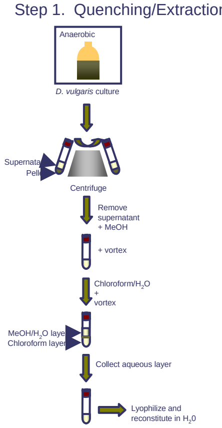
Introduction

The microorganism *Desulfovibrio vulgaris Hildenborough*, because of its metabolic versatility, its ability to remediate heavy metals and radionuclides, coupled with the ease with which it can be maintained in culture is of particular interest to the DOE. However, the effective implementation of remediation strategies and the use of natural attenuation for the cleanup of heavy metal waste in DOE sites is dependent upon understanding critical chemical, physical, and biological processes. Thus, an understanding of regulatory mechanisms and cellular responses to different environmental factors affecting the metal remediation activity *in situ* is of great importance. One approach to study such mechanisms within *D. vulgaris* is to quantify all metabolites within the organism at a given point in time (metabolomics). Since the metabolome is further down the line from gene function, it can reflect more closely the activities of a cell at the functional level than the transcript and the proteome. Capillary electrophoresis and time of flight mass spectrometry (CE-TOFMS) is a promising technique for metabolome research as it provides high separation efficiency and accurate mass determination of compounds with low sample volume requirements. We have utilized novel CE-TOFMS methods for the detection and identification of anionic and cationic metabolites within *D. vulgaris* at mid-log phase. This approach has proven successful in identifying

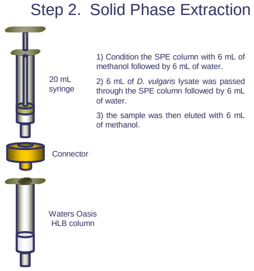
Sample preparation

D. vulgaris cells were inoculated from a log phase culture at 10% (v/v). The cell culture was grown in an anaerobic chamber at 30°C to an OD of ~0.3 (~10^8 cells/ml).

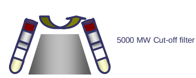
Step 1. Quenching/Extraction



Step 2. Solid Phase Extraction

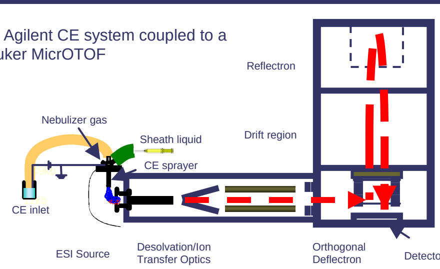

20 mL syringe
1) Condition the SPE column with 6 mL of methanol followed by 6 mL of water.
2) 6 mL of D. vulgaris lysate was passed through the SPE column followed by 6 mL of water.
3) the sample was then eluted with 6 mL of methanol.
Connector
Waters Oasis HLB column

Step 3. Ultrafiltration



CE-TOFMS system

An Agilent CE system coupled to a Bruker MicrOTOF



Strategy for analysis

Transcriptomics ← Pipeline biomass production → Proteomics

↓

Metabolomics

↓

Quenching/Extraction of metabolites

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Sample clean-up by SPE

↓

Separation of metabolites

Cations → CE+ive / MS+ive

Anions → CE+ive / MS-ive

Results

Table 1. Metabolites Identified from *D. vulgaris* lysate

Compound Name	Molecular formula	Theoretical mass
Imidazol	C3H4N2	68.0374
Glycine	C2H5NO2	75.0320
Pyruvic acid	C3H4O3	88.0160
Alanine	C3H7NO2	89.0477
Sarcosine	C3H7NO2	89.0477
Serine	C3H7NO3	105.0426
Cytosine	C4H5N3O	111.0433
Uracil	C4H4N2O2	112.0273
Proline	C5H9NO2	115.0633
Valine	C5H11NO2	117.0790
Betaine	C5H11NO2	117.0790
Succinic acid	C4H6O4	118.0266
Threonine	C4H9NO3	119.0582
Cysteine	C3H7NO2S	121.0197
2-Phenylethylamine	C8H11N	121.0891
Nicotinamide	C6H6N2O	122.0480
Nicotinic acid	C6H5NO2	123.0320
Aminolevulinic acid	C5H9NO3	131.0582
Isoleucine	C6H13NO2	131.0946
Leucine	C6H13NO2	131.0946
Asparagine	C4H8N2O3	132.0535
Ornithine	C5H12N2O2	132.0899
Aspartate	C4H7NO4	133.0375
Homocysteine	C4H9NO2S	135.0354
Adenine	C5H5N5	135.0545
Hypoxanthine	C5H4N4O	136.0385
4-Aminobenzoic acid	C7H7NO2	137.0477
Spermidine	C7H19N3	145.1579
Lysine	C6H14N2O2	146.1055
Glutamate	C5H9NO4	147.0532
Methionine	C5H11NO2S	149.0510
Guanine	C5H5N5O	151.0494
Histidine	C6H9N3O2	155.0695
Orotic acid	C5H4N2O4	156.0171
Carnitine	C7H15NO3	161.1052
Hydroxylysine	C6H14N2O3	162.1004
Phenylalanine	C9H11NO2	165.0790
Pyridoxine	C8H11NO3	169.0739
Aconitic acid	C6H6O6	174.0164
Arginine	C6H14N4O2	174.1117
Citrulline	C6H13N3O3	175.0957
Glucosamine	C6H13NO5	179.0794
Tyrosine	C9H11NO3	181.0739
Spermine	C10H26N4	202.2157
6-aminopenicillinic acid	C8H12N2O3S	216.0569
O-succinyl homoserine	C8H13NO6	219.0743
N-acetylglucosamine	C8H15NO6	221.0899
Cystathionine	C7H14N2O4S	222.0674
2-Deoxycytidine	C9H13N3O4	227.0906
Cytidine	C9H13N3O5	243.0855
DeoxyInosine	C10H12N4O4	252.0859
Glucose-6-phosphate	C6H13O9P	260.0297
2-Deoxyguanosine	C10H13N5O4	267.0968
Inosine	C10H12N4O5	268.0808
Arginosuccinic acid	C10H18N4O6	290.1226
Uridine-5-monophosphate	C9H13N2O9P	324.0359
2-Deoxyguanosine-5-monophosphate	C10H14N5O7P	347.0631
Adenosine-5-monophosphate	C10H14N5O7P	347.0631
Guanosine-5-monophosphate	C10H14N5O8P	363.0580
Xanthosine-5-monophosphate	C10H13N4O9P	364.0420
Thymidine-5-diphosphate	C10H16N2O11P2	402.0229
Cytidine-5-diphosphate	C9H15N3O11P2	403.0182
Uridine-5-diphosphate	C9H14N2O12P2	404.0022
Adenosine-5-diphosphate	C10H15N5O10P2	427.0294
Uridine-5-diphospho-N-acethyl-glucoseamine	C17H27N3O17P2	607.0816
Flavin adenine dinucleotide	C27H33N9O15P2	785.1571
Malonyl CoA	[illegible]	[illegible]

Identification of metabolites can be made possible through accurate mass measurements and empirical formula generation. However, when considering structural isomers, accurate mass measurements alone do not provide conclusive identification of metabolites as several compounds can have the same empirical formula and hence the same molecular mass. In such cases the elution order from CE separation is required for identification with a high degree of confidence. This can be obtained by comparing the elution order of the compound of interest with the chemical standard. Such an approach can be referred to as targeted analysis. The metabolites shown in Table 1 were identified using this methodology.

From the selection of metabolites that we have shown in Table 1 we can gain insights into specific aspects of *D. vulgaris* metabolism. For example, cysteine, serine and homoserine all play a significant role in methionine biosynthesis. Interestingly, O-succinyl homoserine, cystathionine and homocysteine are also intermediates in methionine metabolic pathways. Furthermore the product of glycolysis, pyruvic acid, as well as citric acid cycle intermediates succinic acid and malonyl coenzyme A, were also identified. Products and intermediates of purine and pyrimidine metabolism, products of the urea cycle, as well as intermediates in secondary metabolism were also observed. We are in the process of obtaining standards for the remaining metabolites of the major *D. vulgaris* pathways in order to characterize metabolism in this organism as fully as possible.

The 67 metabolites found in *D. vulgaris* lysate (Table 1) represent only a small fraction of the total metabolite pool. This was further emphasized by the much larger number of unknown compounds that were also observed from *D. vulgaris* lysate (Table 2). By obtaining chemical standards we hope to address the identities of these unknown compounds.

Table 2. Number of metabolites detected from *D. vulgaris* lysate

	Number of metabolites
D. vulgaris targeted	67
D. vulgaris unknown	521
Total	588

Conclusions

Metabolites from most classes of compound, including amino acids, coenzyme As, nucleosides, nucleotides and organic acids were identified by CE-TOFMS.

A total of 588 metabolites were observed from *D. vulgaris* lysate, of which 67 were targeted.

Future work

Utilize more chemical standards to conduct targeted analysis on a wider pool of metabolites from the various metabolic pathways in order to identify these unknown compounds.

To apply our current methodology to stress versus control *D. vulgaris* cultures in order to study the response of this organism to various environmental factors.

ACKNOWLEDGEMENT

ESPP is part of the Virtual Institute for Microbial Stress and Survival supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics Program:GTL through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.